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(54) Title: IMPROVED EXPRESSION VECTOR FOR CONSISTENT CELLULAR EXPRESSION OF THE TET ON REPRESSOR IN MULTIPLE CELL TYPES

(57) Abstract

The present invention provides a vector comprising Protein Translation Peptide Elongation Factor-1 \alpha promoter and nucleic acids encoding reverse tetracycline controlled transactivator, wherein the expression of said transactivator is under the control of Protein Translation Peptide Elongation Factor-1 α promoter. In addition, the invention provides a method of generating a reverse tetracycline controlled transactivator expression system for inducible tetracycline regulated gene expression comprising: (a) isolation of a DNA fragment encoding the reverse tetracycline controlled transactivator by restriction enzyme digestion, (b) generation of Protein Translation Peptide Elongation Factor-1 \(\alpha \) promoter vector, by restriction enzyme digestion, (c) directional cloning of reverse tetracycline controlled transactivator into Protein Translation Peptide Elongation Factor-1 α promoter vector by ligation of 5' EcoRi compatible restriction enzyme overhangs, (d) directional cloning of reverse tetracycline controlled transactivator into Protein Translation Peptide Elongation Factor-1 \alpha promoter vector by Klenow fragment mediated blunt end generation of 3' Bam HI end of DNA fragment encoding the reverse tetracycline controlled transactivator and 3' XbaI end of Protein Translation Peptide Elongation Factor-1 \alpha promoter vector and (e) blunt cloning of partially ligated fragment to produce Protein Translation Peptide Elongation Factor-1 α promoter vector expressing reverse tetracycline controlled transactivator.